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Neozygites species associated with aphids in Chile: current status and new reports

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Abstract —Three species of *Neozygites* were recorded during a 2007–13 survey of the occurrence of the genus on aphids in Chile. *Neozygites osornensis* is known from recent studies, and *N. fresenii* and *N. cinarae* are reported as new records for Chile. *Neozygites lageniformis*, which was not found during the survey, had been recorded previously in Chile. Morphological descriptions, symptoms on infected insects, host spectrum, and geographical distribution of all four species are presented, and a key to *Neozygites* species associated with aphids in Chile is included. These fungi, which are important natural enemies of aphids, may be considered for future aphid pest control.

Key words — entomopathogenic fungi, aphid pathogens, *Entomophthoromycota*

Introduction

Insect-pathogenic fungi represent a phylogenetically heterogeneous group of microorganisms. However, a majority of the economically important species belongs to the phyla *Ascomycota* (order *Hypocreales*) and *Entomophthoromycota*. In general, these fungi are considered excellent candidates for the biological control of various insect pests (Latgé & Papierok 1988, Charnley & Collins 2007, Roy et al. 2010). The hypocrealean species are well studied and some of them are

commercially used with success in various strategies of biological control (Shah & Pell 2003, Sandhu et al. 2012). While species in the *Entomophthoromycota* have been used successfully in the laboratory, they have not yet been commercially formulated into an applicable mycoinsecticide (Barta & Cagáň 2006a), although they have been introduced and spread successfully as an inoculum (Milner et al. 1982, Elkinton et al. 1991). Conservation biological control has been used for other fungi in *Entomophthoromycota,* and there have been attempts in the United States of America to suppress mite populations in soybean crops with fungal pathogens rather than insecticide treatments (Klubertanz et al. 1991).

Neozygites (*Neozygitales*, *Neozygitaceae*) (Humber 2012) comprises a relatively homogenous group of 18 species exclusively pathogenic to arthropods (*Acari*, *Hemiptera*, and *Thysanoptera*) (Delalibera et al. 1992, Montserrat et al. 1998, Shah & Pell 2003, Keller & Petrini 2005, Keller 2007, Wekesa et al. 2010). *Neozygites* species are important natural enemies that may control arthropod populations under microclimatic conditions favorable for the fungus (Latgé & Papierok 1988; Barta & Cagáň 2003, 2006a; Klingen & Westrum 2007; Castro et al. 2013). Generally, most *Neozygites* species can be divided into two subgroups (*Neozygites fresenii*-type or *Neozygites floridana*-type) based on their morphologies and life cycles (Keller 1997, 2006; Delalibera et al. 2004). Their narrow host specificity makes them valuable control agents that present a negligible threat to non-target organisms (Barta 2004). Although the genus has a worldwide distribution, some *Neozygites* species are known only from their type localities. As there is limited literature about the genus in Chile (Aruta et al. 1984; Aruta & Carrillo 1989; Montalva et al. 2010, 2013), our goal was to review and update knowledge on *Neozygites* species associated with aphids in Chile.

Materials & methods

The literature was searched for publications on *Neozygites* associated with aphids in South America with an emphasis on Chile. During 2007–13, aphid populations were observed for *Neozygites* in 25 localities of southern Chile (36°49′41.5″–53°10′00″S 70°56′00″–73°03′04.93″W).

Dead aphids were collected in the field. Black cadavers filled with internally produced resting spores were either examined immediately after being taken to the laboratory or stored at 4 °C until examination. Brown cadavers filled with vegetative cells and hyphal bodies were incubated at 25 °C in moist chambers (plastic containers and moistened filter paper) to induce external conidiogenesis. *Neozygites* species were identified based on morphology according to Keller (1991, 1997).

Mounts were prepared in lactophenol-aceto-orcein (LPAO) as described by Keller (1987). Fungal structures were examined with a Nikon Eclipse

E600 microscope, photographed with a Nikon DS-Fi1 digital camera at a magnification of 1500×, and measured with Motic Images Plus 2.0 software. At least four series of measurements were taken for taxonomically important microstructures. Each series represented 50 measurements, and for each series a mean value was calculated. A final value of each dimension consisted of two ranges: the range of mean values of all series and the range of extreme values of all measurements (in parentheses).

Vouchers of the newly recorded species were conserved in Laboratorio Regional, Servicio Agrícola y Ganadero, Osorno, Chile (LA-N).

Results

Current status of aphid-pathogenic *Neozygites* species in Chile

Neozygites lageniformis was not found during this survey but has been recorded previously in Chile. Two of the three aphid-pathogenic *Neozygites* species observed during the survey are presented as first records from this country. Table 1 provides morphometric characteristics of all *Neozygites* species recorded in Chile. Detailed data on each species are presented below.

Taxonomic descriptions

Neozygites cinarae S. Keller, Sydowia 49: 137. 1997. Plate 1

Hyphal bodies not observed. Primary conidia ovoid to pyriform, formed apically on unbranched conidiophores, with papilla distinct, truncate or slightly rounded, 25.7–26.8 × 18.4–19.5 µm. Forcibly discharged secondary conidia were only rarely observed; capilliconidia are short almond-shaped with a terminal mucoid droplet, brownish, produced apically on slender capillary, 26.0–30.3 × 11.5–12.8 µm. Zygospores ellipsoidal, black, with smooth surface, 33.8–35.3 × 24.3–24.7 µm.

Specimen examined: on *Cinara cedri* Mimeur: **CHILE**, Valdivia, 39°48′18″S 73°15′10″W, March 2013, by C. Montalva (LA-N15032013).

Ecology & occurrence: Infected aphids were found fixed by their probosces on the underside of branches of *Cedrus atlantica* (Endl.) Manetti ex Carrière. Almost all aphids (> 90%) sampled from March to May 2013 were infected and brown or black when filled with resting spores. This is the first record for Chile.

Neozygites fresenii (Nowak.) Remaud. & S. Keller, Mycotaxon 11: 332. 1980. Plate 2

Hyphal bodies spherical to subspherical, 13.0–13.9 µm in diameter. Conidiophores unbranched. Primary conidia spherical, 21.0–21.7 × 15.9–16.3 µm; papilla distinct, cylindrical, blunt to slightly rounded. Secondary conidia (if discharged) like primary conidia on short unbranched conidiophores; capilliconidia passively dispersed, almond-shaped, apical on slender

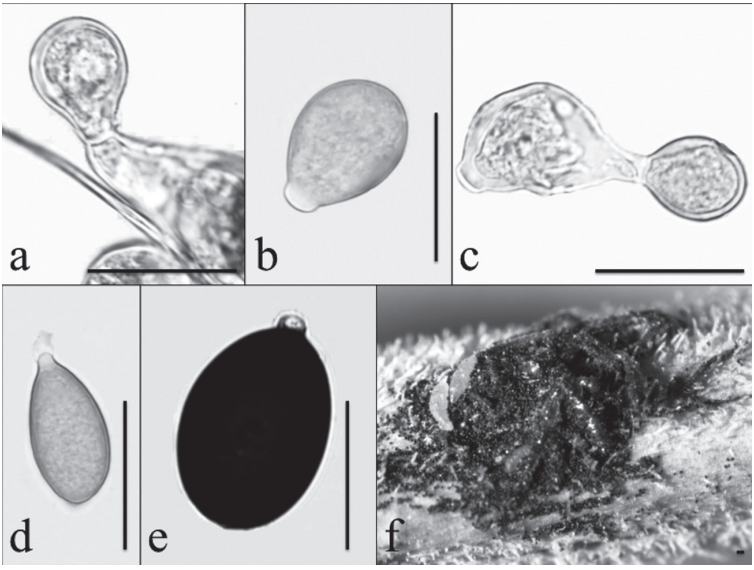

Plate 1. *Neozygites cinarae* on *Cinara cedri*. a. Conidiophore forming primary conidium. b. Primary conidium. c. Formation of secondary conidium. d. Capilliconidium. e. Resting spore. f. *Cinara cedri* infected with *Neozygites cinarae*. Bars = 25 µm.

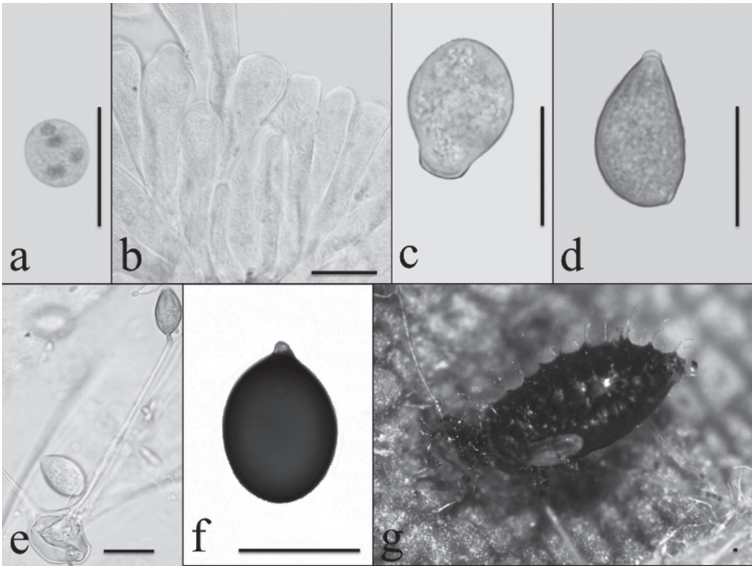

Plate 2. *Neozygites fresenii* on *Myzocallis coryli*. a. Hyphal body with visible nuclei. b. Unbranched conidiophores. c. Primary conidium. d. Capilliconidium. e. Formation of capilliconidia. f. Resting spore. g. *Myzocallis coryli* infected with *Neozygites fresenii*. Bars = 20 µm.

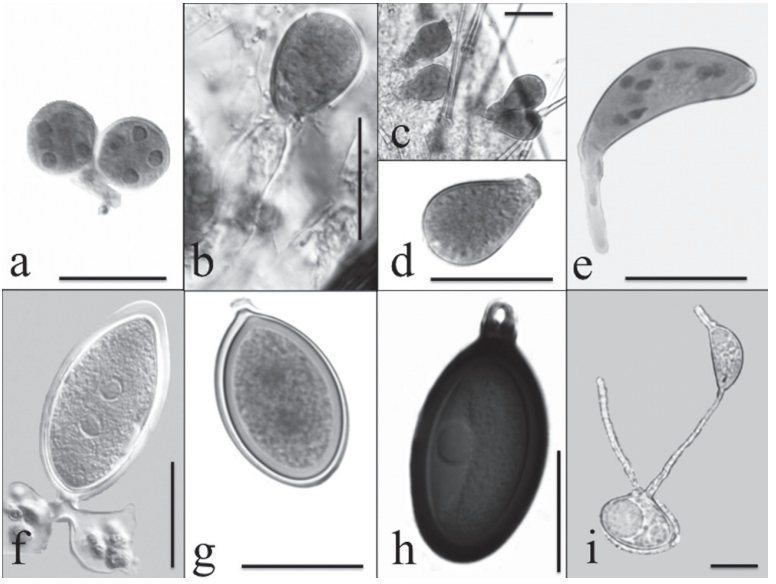

Plate 3. *Neozygites osornensis* on *Cinara cupressi*. a. Subspherical hyphal bodies with visible nuclei. b. Conidiophore forming primary conidium. c. Primary conidia attached to aphid leg. d. Primary conidium. e. Crescent-shaped capilliconidium with visible nuclei. f. Zygospore with visible nuclei developing by conjugation of two hyphal bodies containing degenerating nuclei. g. Immature zygospore. h. Fully developed zygospore. i. Young germ capilliconidium produced by zygospore on long capillary germ conidiophore. Bars = 20 μm.

capilliconidiophores, 22.8–24.3 × 12.8–13.2 μm. Zygospores broadly ellipsoidal, thick-walled, episporium black, smooth, 26.1–28.4 × 18.7–20.4 μm.

Specimens examined: on *Myzocallis coryli* (Goetze): **CHILE**, Puyehue, 40°38′19″S 72°40′55″W, 2012, by C. Montalva (LA-N25032012).

Ecology & occurrence: Infected aphids fixed by their probosces and/or legs were found on the underside of branches of the common hazel (*Corylus avellana* L.); grayish brown to brick or dark brown when conidia were formed, and black when resting spores were present. About 30% of the aphids sampled from February to April 2012 were infected. This is the first record for Chile.

Neozygites osornensis Montalva & Barta, Mycologia 105: 663. 2013. Plate 3

Hyphal bodies spherical or subspherical, 16.4–16.9 μm in diameter. Primary conidia form apically on unbranched conidiophores, ovoid to pyriform, with papilla truncate or slightly rounded, 20.8–23.8 × 13.6–15.9 μm. capilliconidia apical on long slender capillary conidiophores, elongate, crescent-shaped, 28.0–28.5 × 8.5–8.8 μm. Zygospores ellipsoidal, with episporium smooth, dark-brown to black, 31.8–40.8 × 21.5–22.7 μm; germinating by forming capillary

Table 1. Morphometric analysis of taxonomically important structures of four different *Neozygites* species on aphids in Chile.

Fungal structures	*N. cinarae*	*N. fresenii*	*N. lageniformis*	*N. osornensis*
Hyphal bodies (diam.; µm)	No data	13.0–13.9 (4 series)	No data	16.4–16.9 (4 series)
Nuclei (diam.; µm)	No data	2.60–2.84 (4 series)	No data	3.16–3.36 (4 series)
Conidiophores	Unbranched	Unbranched	No data	Unbranched
Primary conidia (µm)				
Length (L)	25.7–26.8 (4 series*)	21.0–21.7 (4 series)	28.4–32.2	20.8–23.8 (4 series)
Diameter (D)	18.4–19.5 (4 series)	15.9–16.3 (4 series)	17.3–21.5	13.6–15.9 (4 series)
L/D	1.4–1.4 (4 series)	1.33–1.36 (4 series)	No data	1.48–1.52 (4 series)
Capilliconidia (µm)				
Length (L)	26.0–30.3 (4 series)	22.8–24.3 (4 series)	30.0–33.6	28.0–28.5 (4 series)
Diameter (D)	11.5–12.8 (4 series)	12.8–13.2 (4 series)	17.7	8.5–8.8 (4 series)
L/D	2.23–2.37 (4 series)	1.79–1.85 (4 series)	No data	3.23–3.36 (4 series)
Number of nuclei	4 (3–6) (n= 7**)	No data	No data	7 (5–10) (n= 20)
Capillary tube length (µm)	No data	No data	No data	30.5–31.8 (2= series)
Resting spores (µm)				
Length (L)	33.8–35.3 (4 series)	26.1–28.4 (4 series)	No data	31.8–40.8 (4 series)
Diameter (D)	24.3–24.7 (4 series)	18.7–20.4 (4 series)	No data	21.5–22.7 (4 series)
L/D	1.37–1.43 (4 series)	1.39 (4 series)	No data	1.47–1.85 (4 series)
Rhizoids	Absent	Absent	Absent	Absent
Reference	This paper	This paper	Aruta & Carrillo 1989	Montalva et al. 2013

* Series = one series represents 50 measurements; the range consists of mean extreme values of series.

**n = number of measurements.

conidiophores, averaging 72.4 µm long (n: 43), with apical germ capilliconidia, 28.0 × 12.4 µm (n: 45) (Montalva et al. 2013).

Specimens examined: on *Cinara cupressi*: **CHILE**, Osorno, 40°35′14″S 73°05′38″W, 4 April 2007, by C. Montalva (SGO 161 401, holotype).

Ecology & occurrence: Infected aphids can be easily recognized hanging on twigs and by an obvious change of their color during sporulation (turning from yellowish-brown to reddish-brown). During the infection progress, aphids gradually became swollen and turgid. Cadavers that were reddish-brown during the formation of external conidia became black when internal zygospores developed. Cadavers usually hung and remained with their probosces inserted in the branches. After certain time the cadavers either dried up and fell down to the soil or liquefied and disintegrated during zygosporogenesis, thus dispersing the resting spores on the bark of twigs. More than 80% of aphids sampled from November to December 2013 in Osorno were infected (Montalva et al. 2013).

Key to aphid-pathogenic *Neozygites* species from Chile

1. Observed on *Myzocallidinae* or *Aphidinae* 2
1. Observed on *Lachninae* or *Pterocommatinae* 3
2. Primary conidia average length ≤22 µm; capilliconidia averaging < 25 µm, with L/D 1.8. . . . . *Neozygites fresenii*
2. Primary conidia average length ≥29 µm; capilliconidia averaging ≥ 30 µm, with L/D 2.0. . . . . *Neozygites lageniformis*
3. Primary conidia average length 26 µm; capilliconidia almond-shaped, average diameter >11 µm. . . . . *Neozygites cinarae*
3. Primary conidia average length 23 µm; capilliconidia crescent-shaped, average diameter <9 µm. . . . . *Neozygites osornensis*

Discussion

Dead insects and mites killed by different *Neozygites* species have been reported from several South American countries (Humber et al. 1981, Agudelo-Silva 1986, Löhr et al. 1990, Gómez de Picho 1991, Delalibera et al. 1997, 2000, Van der Geest et al. 2002, Scorsetti & López-Lastra 2007, Roggia et al. 2009). Previously, however, *Neozygites* species pathogenic to aphids have been recorded only in Argentina and Chile (Scorsetti et al. 2007, Sosa-Gómez et al. 2010, Montalva et al. 2013). The first reports from Chile of any *Neozygites* species were made in the 1980s, when *N. parvispora* (D.M. MacLeod & K.P. Carl) Remaud. & S. Keller (on thrips) and *N. lageniformis* (on aphids) were observed infecting natural arthropod populations (Aruta et al. 1984, Aruta & Carrillo 1989). *Neozygites parvispora* is a widespread fungal parasite of various thrips species with a primarily European distribution (Bałazy 1993, Keller 1997). *Neozygites lageniformis* is rare and incompletely described species from the

New World (Thaxter 1888, Aruta & Carrillo 1989), previously observed only twice (in aphids in United States of America and Chile). During the survey we did not find this fungus in *Myzocallis coryli* colonies. Although there are no complete data available on its morphology, *N. lageniformis* is still regarded as a separate species (Bałazy 1993). However, until additional records and a more accurate description become available, its taxonomic status is uncertain.

Neozygites cinarae is a fungal pathogen of aphids in the genus *Cinara*. Previously reported from Europe on *C. pilicornis* (Hartig) and *C. piceae* (Panzer) (Keller 1997, Barta et al. 2005, Barta & Cagáň 2006b, Montalva et al. 2013), ours is first report for South America. In Chile, *N. cinarae* was found only in *C. cedri* colonies on the host tree, *Cedrus atlantica*. No data are available about its use as a biological control in laboratory or field conditions. Its high incidence during our survey suggests that during autumn *N. cinarae* could be a good natural regulator of *C. cedri* populations along with other natural enemies such as coccinellid beetles and syrphid flies.

Neozygites fresenii is a widely occurring fungal pathogen of aphids. It has been reported from Europe (Keller 1991, Bałazy 1993, Barta & Cagáň 2002), North and South America (Steinkraus et al. 1995, Scorsetti et al. 2007), South Africa (Hatting et al. 1999), the South Pacific (Keller 1997), and Australia (Milner & Holdom 1986). Although the fungus is reported to have a broader host spectrum, in Chile *N. fresenii* was found only in *Myzocallis coryli* colonies. In Argentina, *N. fresenii* has also been identified from *Brevicoryne brassicae* L., *Myzus* sp., and *Aphis fabae* Scop. in Buenos Aires province (Scorsetti et al. 2007). *Neozygites fresenii* often causes widespread epizootics in aphid populations (Steinkraus et al. 1995, Keller 1997, Barta 2004). While this fungus is considered to be best adapted to hot, humid, and even tropical conditions (Remaudière 1977, Keller 1997), epizootics have also been reported in Iceland (Austarå et al. 1997, Nielsen et al. 2001). The epizootiology of *N. fresenii* in annual crop ecosystems has been well studied (Steinkraus et al. 1995, 1996, Barta & Cagáň 2002), but its commercial exploitation is limited due to absence of in vitro isolates. In Arkansas (USA), a method for mass-harvesting *N. fresenii* from natural epizootics in *Aphis gossypii* populations in a commercial cotton field was developed with the intention of using the collected inoculum for targeted aphid control (Steinkraus & Boys 2005).

Neozygites osornensis, first described during a survey of natural enemies of invasive *Cinara cupressi* colonies in Chile (Montalva et al. 2013), was also found in five localities in southern Chile on *C. cupressi* and *C. tujafilina*, which usually co-occurs with *C. cupressi*. The infection usually occurred between November (end of spring) and March (end of summer). We suggest that *N. osornensis* is a natural enemy that could serve as a biological control of *C. cupressi* in Chile. *Neozygites osornensis* might also be a candidate for conservation biological

control of *C. tujafilina,* an aphid attacking a native host tree, *Austrocedrus chilensis* (D. Don) Pic. Serm. & Bizzarri, whose populations are now defined as vulnerable (Hechenleitner et al. 2005). The application of chemical insecticides to control *C. tujafilina* is forbidden in national parks (Corporación Nacional Forestal, CONAF) without permission (CONAF 2006).

All *Neozygites* species that we found in Chile produce resting spores that allow the fungi to persist during unfavorable conditions for several years (Hajek 1997) and, thereby, to serve as a reservoir of infection in the ecosystem. For *Neozygites* collected during the surveys, we usually found capilliconidia on aphid cadavers diseased by *N. cinarae* and *N. fresenii* during March (end of summer). On the other hand, *N. osornensis* capilliconidia were less frequently observed and were obtained only in the laboratory in a humid chamber on infected *Cinara cupressi* collected in Osorno during November (end of spring). Capilliconidia of *Neozygites* spp., which are usually produced during the vegetation period, are responsible for disseminating infection among host populations (Keller 1997). Contrary to what Keller reported (1997), we observed forcibly discharged secondary conidia of *N. cinarae* in Valdivia, although this conidial type was found only rarely. The absence of rhizoids and pseudocystidia for all species discussed here agrees with Keller (1991, 1997) and Bałazy (1993). Very few studies have been published for any location treating natural occurrences of entomophthoroid fungi infecting aphids feeding on woody plants. However, it appears that *N. fresenii*, *Entomophthora planchoniana* Cornu, and *Zoophthora aphidis* (Hoffm.) A. Batko are the most frequent fungal species associated with tree-dwelling aphids (Nielsen et al. 2007, Barta 2009); these findings are reinforced by our findings about *N. fresenii*.

The identification of these fungi is an important step for a better knowledge of natural fungal enemies of aphids in Chile and throughout the world. However, more studies are on virulence, culturing, strain characterization, and molecular identification are needed to bring greater and much needed clarity to our understanding of these relatively little known fungi.

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